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The relative importance of sediment and water column supplies of nutrients to the growth and tissue nutrient content of the green macroalga *Enteromorpha intestinalis* along an estuarine resource gradient

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Abstract

Large blooms of opportunistic green macroalgae such as *Enteromorpha intestinalis* are of ecological concern in estuaries worldwide. Macroalgae derive their nutrients from the water column but estuarine sediments may also be an important nutrient source. We hypothesized that the importance of these nutrient sources to *E. intestinalis* varies along a nutrient-resource gradient within an estuary. We tested this in experimental units constructed with water and sediments collected from 3 sites in Upper Newport Bay estuary, California, US, that varied greatly in water column nutrient concentrations. For each site there were three treatments: sediments + water; sediments + water + *Enteromorpha intestinalis* (algae); inert sand + water + algae. Water in units was exchanged weekly simulating low turnover characteristic of poorly flushed estuaries. The importance of the water column versus sediments as a source of nutrients to *E. intestinalis* varied with the magnitude of the different sources. When initial water column levels of dissolved inorganic nitrogen (DIN) and soluble reactive phosphorus (SRP) were low, estuarine sediments increased *E. intestinalis* growth and tissue nutrient content. In experimental units from sites where initial water column DIN was high, there was no effect of estuarine sediments on *E. intestinalis* growth or tissue N content. Salinity, however, was low in these units and may have inhibited growth. *E. intestinalis* growth and tissue P content were highest in units from the site with highest initial sediment nutrient content. Water column DIN was depleted each week of the experiment. Thus, the water column was a primary source of nutrients to the algae when water column nutrient supply was high, and the sediments supplemented nutrient supply to the algae when water column nutrient sources were low. Depletion of water column DIN in sediment + water units indicated that the sediments may have acted as a nutrient sink in the absence of macroalgae. Our data provide direct experimental evidence that macroalgae utilize and ecologically benefit from nutrients stored in estuarine sediments.

Introduction

Large blooms of opportunistic green macroalgae such as *Enteromorpha* and *Ulva* spp. occur in estuaries throughout the world (e.g., Sfriso et al. 1987, 1992; Schramm and Nienhuis 1996; Raffaelli et al. 1999) often in response to increased nutrient loads from developed watersheds (Valiela et al. 1992; Nixon 1995; Paerl 1999). While these algae are natural components of estuarine systems and play integral roles in

estuarine processes (Pregnall and Rudy 1985; Kwak and Zedler 1997; Boyer 2002), blooms are of ecological concern because they can reduce the habitat quality of an estuary. They can deplete the water column and sediments of oxygen (Sfriso et al. 1987, 1992) leading to changes in species composition, shifts in community structure (Raffaelli et al. 1991; Ahern et al. 1995; Thiel and Watling 1998), and loss of ecosystem function.

Growth of *Enteromorpha* and *Ulva* spp. is often regulated by nutrient availability (Sfriso et al. 1987; Hernández et al. 1997; Schramm 1999). These algae efficiently remove nitrogen (N) from the water column (Fujita 1985; O'Brien and Wheeler 1987; Duke et al. 1989a, 1989b). In estuaries, N levels are generally higher near the head of the system, where rivers flow in, and decrease toward the mouth or the opening to the ocean (Rizzo and Christian 1996; Hernández et al. 1997; Nedwell et al. 2002). Therefore, the availability of water column N to macroalgae usually decreases along a spatial gradient within an estuary.

Estuarine sediments may also be a significant source of nutrients to macroalgae. The release of nitrogen and phosphorus (P) from sediments is well established (e.g., Boynton et al. 1980; Nixon 1981; Clavero et al. 2000; Grenz et al. 2000). Flux of nutrients from sediments is believed to increase availability of water column nutrients to primary producers; a number of studies have constructed nutrient budgets in which N and P fluxing from sediments may potentially meet a portion of the nutrient requirement of the system's primary producers (Boynton et al. 1980; Blackburn and Henriksen 1983; Trimmer et al. 1998, 2000). However, these studies infer primary producers' use of nutrients fluxing from sediments rather than providing direct evidence.

Several field studies provide correlative evidence that macroalgae take up nutrients fluxing from estuarine sediments. Birch et al. (1981) found that macroalgal tissue nutrient content varied with sediment nutrient content and inferred that nutrient exchange between the sediments and the algae occurred. Thybo-Christesen et al. (1993) showed decreases in water column N and P from the sediment surface toward floating algal mats, indicating uptake by the macroalgae of nutrients fluxing from the sediments. These studies indirectly support the hypothesis that macroalgae utilize nutrients fluxing from sediments.

Several laboratory studies have demonstrated the ability of algal mats to intercept nutrients fluxing from sediments. McGlathery et al. (1997) simulated benthic nutrient flux by using nutrient-enriched seawater in the bottom compartment of an incubation chamber separated from the overlying water by a 0.7 μm filter paper to simulate sediments. Tyler et al. (2001) measured uptake by *Ulva lactuca* of urea released from estuarine sediments; uptake was measured over a 12 h period and longer-term ecological effects such as tissue nutrient status and growth were not assessed. These studies confirm macroalgal uptake of nutrients fluxing from artificial and estuarine sediments, yet there is still a need for study of the effect of sediment nutrients on the long-term ecology of macroalgae.

In tropical work from Kaneohe Bay, Hawaii, Larned and Stimson (1996) substantiated that *Dictyosphaeria cavernosa* utilized sediment-derived N and increased growth resulted. Of the few studies we have found to date documenting a long-term effect of sediments on macroalgal biomass, only one of these is estuarine: Lavery and McComb (1991) found that estuarine sediments from the Peel-Harvey estuary in western Australia increased growth of *Chaetomorpha linum* over a 3-week period. Sediments, however, from different depths were homogenized to reconstruct sediment profiles. Therefore, ecological effects of nutrients fluxing from undisturbed estuarine sediments to macroalgae have not been determined.

The importance of sediments as a source of nutrients to macroalgae is critical in understanding nutrient dynamics in estuaries and factors controlling algal blooms (Valiela et al. 1997). The role of estuarine sediments as a source of nutrients to macroalgae may be particularly important in systems where nutrient inputs are episodic and availability of nutrients in the water column fluctuates over short time scales (Litaker et al. 1987; Day et al. 1995) or where strong spatial gradients in water column nutrient availability exist (Rizzo and Christian 1996; Nedwell et al. 2002). Furthermore, the potential contribution of nutrients from sediments to macroalgae may be significant in poorly-flushed estuaries where water circulation is much reduced due to physical modifications to the system (Fong and Zedler 2000). The installation of culverts and tidal gates often restrict flow, creating areas that only experience circulation when tidal amplitude reaches a threshold (A. Armitage pers. comm.) or preventing drainage of some areas (Fong and Zedler 2000). In such cases, water column nutrients

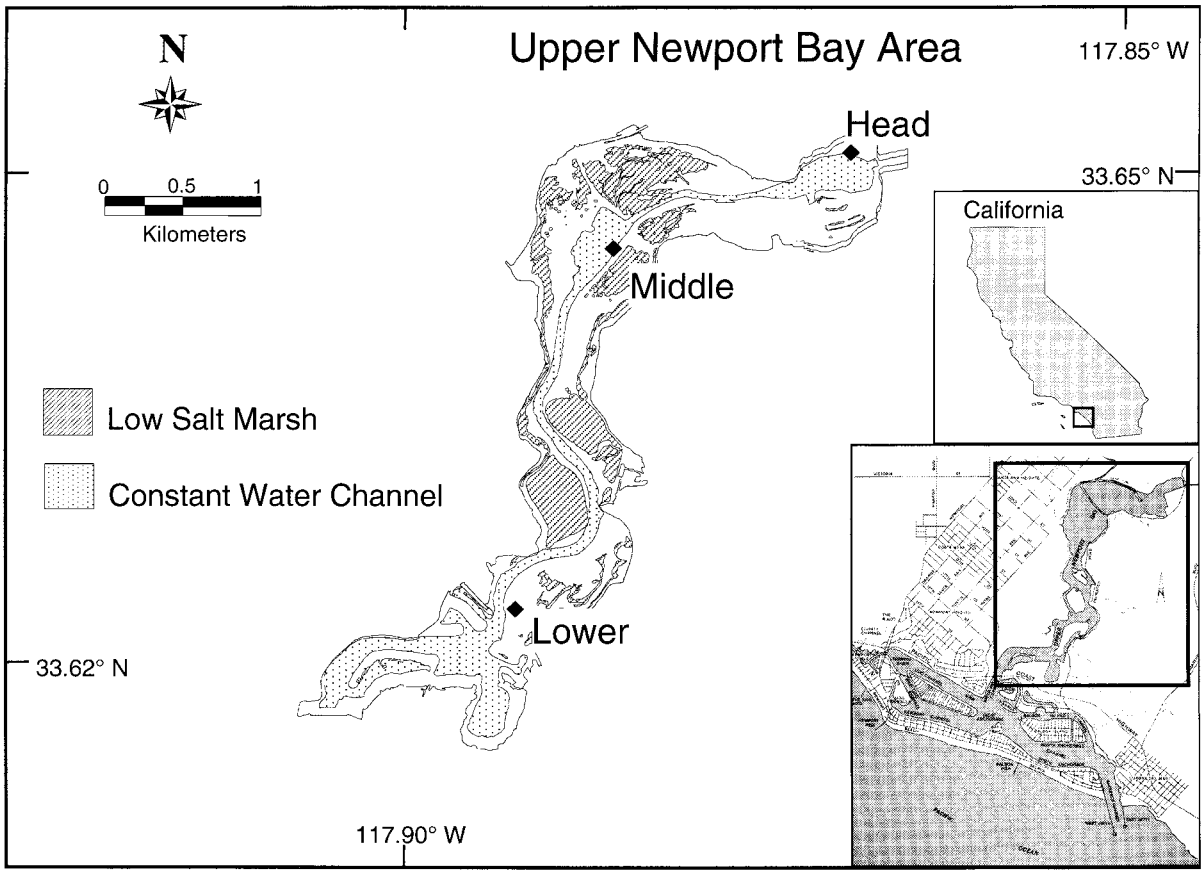

Figure 1. Map of Upper Newport Bay estuary, California, US, with 3 sites (Lower, Middle, and Head) from which water and sediments were collected to construct experimental units.

in stagnant, ponded areas may be depleted by algal uptake much faster than in areas with increased circulation where water column nutrients are continuously supplied. Further work is needed to understand the ecological significance of sediment nutrient efflux to macroalgae across a range of water column nutrient concentrations.

Our objective was to determine the relative importance of the water column and the sediments as sources of nutrients to macroalgae along a gradient of resource availability. We hypothesized that sediment sources will become more important to macroalgae when water column nutrient levels are low, such as may happen at the seaward end of a nutrient gradient within an estuary or in poorly-flushed, ponded areas. Our experimental study modeled Upper Newport Bay (UNB), a large southern California estuary subject to blooms of *Enteromorpha intestinalis* and *Ulva expansa* (Kamer et al. 2001). In UNB, water column nutrient concentrations are consistently high near the head of the estuary (158-800 μM NO_3, 4.3-16.7 μM total P) relative to down-estuary areas (5-90 μM NO_3, 1.8-11.5 μM total P) (Boyle 2002), while sediment nutrient concentrations vary throughout the estuary (0.034-0.166 % dry wt total Kjeldahl nitrogen [TKN], 0.044-0.072 % dry wt P) with no clear spatial pattern (Boyle 2002). UNB also exemplifies estuaries with altered hydrology due to physical modifications. The highly developed and modified Lower Bay separates the natural portion of the estuary (UNB, Figure 1) from the Pacific Ocean, and the permanently established mouth alters the hydrodynamics from those that would occur with a naturally migrating mouth. Although the Upper Bay is largely natural, there are several major berms (just above Middle Site, Figure 1) that reduce circulation and create ponded areas.

Methods

Experimental overview

To test the relative importance of the water column vs. sediments as a source of nutrients to macroalgae, we collected sediment cores and water from 3 sites in UNB along a water column nutrient gradient (Figure 1). Using sediment and water from each site, we constructed three sets of experimental units varying in complexity: sediment + water; sediment + water + *Enteromorpha intestinalis* (algae); inert sand + water + algae. The sediment + water cores served to eliminate algae as a nutrient source/sink, and the inert sand + water + algae cores served to eliminate sediments as a nutrient source/sink. Quantifying nutrients in each component of the experimental system at the beginning and end of the experiment allowed us to determine nutrient allocation among these compartments under different nutrient supply conditions.

Collection of sediment and water

The site nearest the head of the estuary (Head, Figure 1) was at the mouth of San Diego Creek, a large freshwater and nutrient source to UNB. This site had the lowest salinity, the greatest water column DIN, mostly in the form of NO_3, the lowest TKN (all forms of dissolved N except NO_3 and NO_2) and soluble reactive phosphorus (SRP), and was intermediate in terms of sediment P, sand, and silt content (determined according to Bouyoucos 1962) (Table 1). The second site (Middle) was mid-way between the head and the seaward end of the estuary and had intermediate salinity and water column DIN, the highest initial sediment P, the least sand, and the highest silt content (Table 1). The third site (Lower) was just above the transition zone between the natural estuarine habitat of UNB and the highly modified and developed Lower Bay situated at the lower boundary of the estuarine system (Kamer et al. 2001). This site had the highest salinity, the lowest water column DIN, TKN and SRP concentrations similar to the Middle site, and it had the sandiest sediments, the least silt content, and the lowest sediment P (Table 1). All three sites had similar clay content and there was a non-significant trend toward greatest sediment N at the Middle site (Table 1).

At each site we took 10 individual sediment cores to a depth of 8 cm from exposed intertidal mudflats. The top 2–8 mm of each core was oxic, and below

Table 1. Salinity, mean initial water column and sediment nutrients, and sediment grain size distribution from 3 sites in Upper Newport Bay and inert sand used in sand + water + algae treatments. For each variable, significant differences between sites ($p < 0.05$, Fisher's LSD following significant 1-factor ANOVA) are indicated by different superscripts. For water column nutrient data, n=9; means were calculated from triplicate samples from weeks 1, 2, and 3. For sediment data, n=5. Inert sand was not included in nutrient content statistical analyses, as N and P were below detection limits, or grain size statistical analyses, as data were invariant. Values given are means with standard errors between brackets except for salinity (n=1).

	Water column nutrients (μM)					Sediment nutrients (% dry weight)			% Sediment type		
Site (Salinity)	NO_3	NH_4	DIN	TKN	SRP	Total N	Total P	N:P (Molar)	Sand	Silt	Clay
Head (8 psu)	414 (8.0)^a	11.4 (2.3)^a	422 (5.8)^a	33.3 (6.6)^a	< 1.61	0.066 (0.004)^a	0.050 (0.000)^a	2.92	61 (3)^{a,b}	25 (2)^a	14 (1)^a
Middle (25 psu)	101 (3.4)^b	21.0 (0.5)^b	122 (3.4)^b	61.9 (4.3)^b	5.95 (2.37)^a	0.072 (0.002)^a	0.058 (0.002)^b	2.76	53 (3)^b	32 (2)^b	15 (1)^a
Lower (30 psu)	49 (1.8)^c	23.2 (3.7)^b	72 (4.5)^c	55.6 (6.9)^b	3.41 (0.19)^a	0.062 (0.006)^a	0.042 (0.004)^c	3.28	69 (1)^a	18 (1)^c	13 (1)^a
Inert sand	–	–	–	–	–	< 0.05	< 0.01	–	96 (0)	1 (0)	3 (0)

this, the cores were anoxic. Cores were taken in a row parallel to the water line using polycarbonate tubes of 7.3 cm internal diameter × 20 cm length. We used the edge of the vegetation as an elevational guide to ensure sampling of similar elevation among sites. Bottoms of the cores were capped and sealed in the field; tops were left open. Care was taken not to disturb the vertical stratification of the cores. Water was collected at each site from 0.5–1 m depth using a battery operated pump.

Experimental units

In the laboratory, unfiltered water from the corresponding site was added to each polycarbonate tube containing an estuarine sediment core to construct the sediment + water portion of the experimental units. Enough water was added so that 300 ml overlaid each sediment core. The bottom 8 cm of each tube was wrapped with duct tape to block light from entering the sediments through the sides of the tube. To complete the experimental units, we added *Enteromorpha intestinalis* (collected from a single site in Mugu Lagoon, Ventura County, CA, 10 d prior to the initiation of the experiment) to 5 tubes from each site containing estuarine sediments. *E. intestinalis* was placed in nylon mesh bags and spun in a salad spinner for one min to remove excess water. Algae were weighed and 5.0 ± 0.1 g sub-samples were added to experimental units designated as "+ algae" treatments. Initial tissue N was 1.19 ± 0.02 % dry wt (n = 5, mean ± SE) and initial tissue P was 0.11 ± 0.00 % dry wt (n = 5).

To separate the contribution of nutrients to macroalgae from the estuarine sediment and the contribution from the water column, we constructed 5 inert sand + water + algae experimental units per site. These were identical to the sediment + water + algae units with the exception of an 8 cm deep layer of sand in the bottom of each instead of estuarine sediment. The sand was prepared by heating in a muffle furnace to 400 °C for 10 h to remove any organic material, then washing the cooled sand in dilute acid (3% HCl in de-ionized water). The sand was then rinsed of acid and dried to a constant weight at 60 °C in a forced air oven. Initial N of the sand was below the detection limit of 0.05 % dry wt, and P was below the detection limit of 0.01 % dry wt (Table 1). The sand simulated the physical presence of the estuarine sediments, yet had no measurable nutrients to contribute to the macroalgae. This treatment allowed us to compare the response of the algae with sediments and water to the response of algae with nutrient-free sand and water, thereby determining the effects of the sediments on algae.

Experimental units were placed outdoors in a temperature controlled water bath (20 ± 2 °C) and a layer of window screening was used to reduce incident light (2200-2500 μmoles m^{-2} s^{-2} at mid-day) by ~30% to simulate coastal levels (1405-1956 μmoles m^{-2} s^{-2}, Arnold and Murray 1980). Treatments were arranged in a randomized matrix. There were three treatments (sediments + water; sediments + water + algae; inert sand + water + algae) for each of the three sites (Head, Middle, Lower) with 5-fold replication for a total of 45 experimental units. The experiment ran for three weeks. During this time, salinity was monitored with a hand-held refractometer and de-ionized water was added to compensate for evaporation.

Measured responses

At the end of each week, we sampled the water in each experimental unit for nutrients. Algae were removed from the units and, with a 60 cc syringe, we removed all of the water from each unit, except for a thin layer (2–5 mm) overlying the core. Care was taken to ensure that the core surface was not visibly disturbed. A sub-sample of the water removed from each unit was filtered through glass fiber filters (Whatman GF/C), frozen, and analyzed for NO_3 + NO_2 (referred to as NO_3), NH_4, TKN, and SRP. Water levels in two units, each in different treatments, indicated that water was leaking out and these units were excluded from analyses. NO_3 was reduced to NO_2 via cadmium reduction and measured spectrophotometrically after diazotation (Switala 1999; Wendt 1999). NH_4 was heated with solutions of salicylate and hypochlorite and determined spectrophotometrically (Switala 1999; Wendt 1999). TKN was determined by the wet oxidation of nitrogen using sulfuric acid and digestion catalyst. The procedure converts organic nitrogen to NH_4, which is subsequently determined (Carlson 1978). SRP was determined spectrophotometrically following reaction with ammonium molybdate and antimony potassium under acidic conditions (APHA 1998). These automated methods have detection limits of 3.57 μM for N and 1.61 μM for P.

At the end of weeks 1 and 2, we refilled each unit with 300 ml of water from each site that was collected

at the beginning of the experiment. The water was stored in the dark at 6 °C and triplicate samples of water from each site were analyzed weekly for nutrient concentrations. Overall concentrations of NO_3, NH_4, and SRP in the collected and stored water did not vary week to week ($p > 0.05$, ANOVA). TKN increased ($p = 0.002$) over the course of the experiment from 34.92 ± 4.52 initially to 48.41 ± 8.40 μM at the end of week 1 and 67.46 ± 2.94 μM at the end of week 2. Water was added such that it did not disturb the sediment surface. The weekly exchange of water in all units simulated low turnover in poorly-flushed estuaries characteristic of southern California (Zedler 1982; Zedler 1996; Fong and Zedler 2000). Algae were replaced in the appropriate units, and the units were re-randomized in the water bath.

At the end of the experiment, growth of *Enteromorpha intestinalis* was determined by wet weighing the algae and calculating the % change from initial wet weight. Samples were individually rinsed briefly in freshwater to remove external salts, dried in a forced air oven at 60 °C to a constant weight, and re-weighed. Samples were ground with mortar and pestle and analyzed for tissue N and P. N was determined using an induction furnace and a thermal conductivity detector (Dumas 1981). P was determined by atomic absorption spectrometry (AAS) and inductively coupled plasma atomic emission spectrometry (ICP-AES) following a nitric acid/hydrogen peroxide microwave digestion (Meyer and Keliher 1992). N and P content of algae are reported as total mass unit^{-1}, which is calculated by multiplying the nutrient concentration of a sample (% dry weight) as a proportion by the dry weight of that sample:

mg N or P unit^{-1} = [% tissue N or P/100] * dry wt (g) * 1000 mg/g

Each sediment or sand core was removed from its unit at the end of the experiment and homogenized. One-third of the amount of each homogenized core was randomly selected and dried in a forced air oven at 60 °C to a constant weight, ground with mortar and pestle and analyzed for N and P. N was determined by use of a dynamic flash combustion system coupled with a gas chromatographic separation system and a thermal conductivity system (Dumas 1981). P was determined by AAS and ICP-AES following a nitric acid/hydrogen peroxide microwave digestion (Meyer and Keliher 1992). Final sediment N and P are reported as % change from initials.

Data analysis

All data were tested for normality and homogeneity of variance. Non-normal sediment P values were transformed by adding a constant and taking the square root of the sum. Among treatment differences in *Enteromorpha intestinalis* growth and tissue N and P total mass unit^{-1} were analyzed using 2-factor ANOVA (site × core material, where core material was either estuarine sediment or inert sand). Among treatment differences in final sediment N and P content were analyzed using 2-factor ANOVA (site × algae, where algae was either present or absent). Final sand N and P values were not analyzed statistically as they were all below the detection limits of 0.05 % dry wt and 0.01 % dry wt respectively.

NO_3 and NH_4 values of water removed from each experimental unit at the end of each week were often below the detection limit of 3.57 μM. SRP values from the end of weeks 1 and 2 were often below the detection limit of 1.61 μM. Statistical analyses of the remaining values were not conducted due to low sample size. Differences in water column SRP at the end of the third week were analyzed using 2-factor ANOVA (site × treatment, where treatment was either estuarine sediment only, estuarine sediment + algae, or inert sand + algae). TKN data from the end of each week was analyzed using 2-factor repeated measures ANOVA (site × treatment × time). Unless otherwise stated, no significant interactions occurred between factors in ANOVA.

Results

Enteromorpha intestinalis grew faster when incubated with estuarine sediments than with sand (Figure 2). This effect appeared to be strongest in Middle and Lower site treatments, where water column DIN levels were lower than in treatments from the Head, which did not differ for sediment versus sand. Growth in these latter units was low, possibly due to low salinity conditions. *E. intestinalis* grew most (41%) in units containing estuarine sediments from the Middle site where initial sediment nutrients were highest. Algal biomass grew only 12-16% in 3 weeks when incubated with sand. The significant interaction between the site and core material was probably due to differences between +sediment units from different sites and a lack of differences among +sand units from different sites.

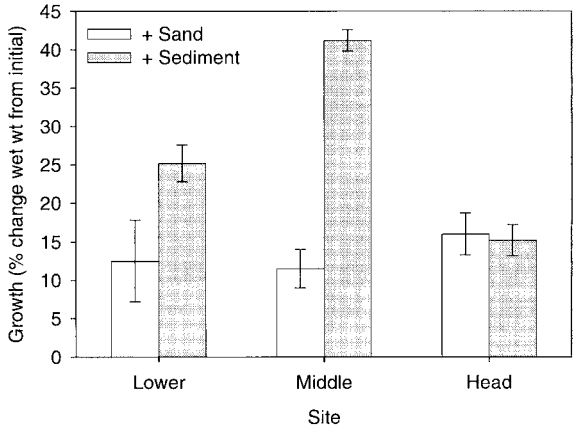

Figure 2. Enteromorpha intestinalis biomass (as % change from initial) grown with either inert sand or estuarine sediment from 3 sites in Upper Newport Bay. Bars represent ± 1 SE. Two-factor ANOVA summary statistics: site: $p = 0.003$; core material: $p = 0.001$; interaction: $p = 0.001$.

Enteromorpha intestinalis tissue N and P content (total mass unit^{-1}) at the end of the experiment was higher in +sediment units compared to +sand (Figure 3, A and B). For N, this effect was apparent in Lower and Middle site treatments but not in treatments from the Head. Overall, tissue N was greatest in treatments from the Head and decreased with distance down-estuary, tracking water column patterns. The effect of estuarine sediment on algal tissue P was evident in treatments from all three sites. When sediments were present, tissue P was greatest in units from the Middle site.

Water column N supplies were greatly reduced in all units each week of the experiment (Table 2). At the end of the first week, the NO_3 remaining in the sediment + water treatments from the Head was ~91% less than the initial values from this site. Water column TKN was significantly affected by time ($p = 0.001$) but not by site ($p = 0.172$) or treatment ($p=0.922$). The range of mean TKN for all treatments at the end of each week (Table 3) encompassed the initial grand mean of all sites of 34.92 ± 4.52 μM.

Phosphorus availability in the water column was low in many units during the first two weeks of the experiment (Table 4). SRP was below detection limit in all units from the Head and many algae-containing units from the Middle and Lower sites during this time. It was higher in sediment-only units from the latter two sites. Water column P availability was greater during the final week of the experiment; where comparisons are possible, values were generally higher in the third week and far less samples were below detection limit. SRP at the end of the third week was significantly affected by site ($p = 0.011$) but not treatment ($p = 0.512$). SRP in units from the Head appear to be the lowest (Table 4).

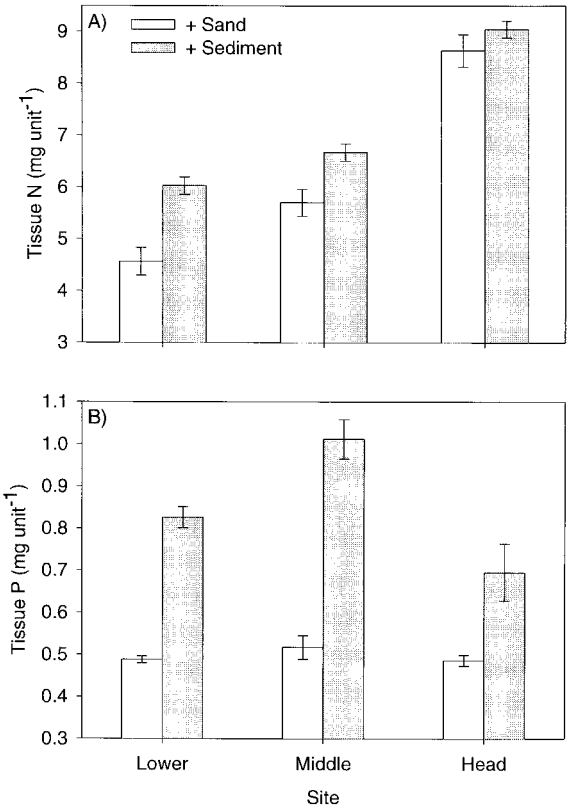

Figure 3. Mass of nitrogen (A) and phosphorus (B) in *Enteromorpha intestinalis* tissue grown with either inert sand or estuarine sediment from 3 sites in Upper Newport Bay. Bars represent ± 1 SE. Two-factor ANOVA summary statistics for nitrogen: site: $p = 0.001$; core material: $p = 0.001$; and for phosphorus: site: $p = 0.001$; core material: $p = 0.001$; interaction: $p = 0.006$.

Final sediment N (% change from initial) was highly variable (Figure 4A). There were no significant differences among sites but there was a trend of increased N over the course of the experiment in units from the Lower and Middle sites. Sediment P increased in units from the Lower site but decreased in units from the Middle site during the experiment (Figure 4B). In units from the Head, sediment P tended to increase when algae were not present but not when algae were present.

Table 2. Inorganic nitrogen concentrations (μM) at the end of each week of the experiment. If all samples within a treatment were < 3.57 μM, the notation "BDL" (Below Detection Limit) is used. In samples with values below and above the detection limit, only the latter values are given. Sample size is noted.

Site	Week 1		Week 2		Week 3	
	NO_3	NH_4	NO_3	NH_4	NO_3	NH_4
Head	37.29 ± 12.54 (n = 5) Sediment only	BDL	BDL	BDL	5.00 (n = 1)	5.71 (n = 1)
Middle	BDL	BDL	BDL	BDL	BDL	BDL
Lower	BDL	BDL	BDL	5.71 (n=1)	BDL	BDL

Table 3. Total Kjeldahl nitrogen (TKN) concentrations (μM) at the end of each week of the experiment. Grand means (n = 43) are presented as neither site nor treatment effect was significant. Values presented are means ± 1 SE.

Week	TKN
1	40.03 ± 1.54
2	26.08 ± 1.38
3	30.23 ± 1.38

Discussion

The importance of the water column versus sediments as sources of nutrients to support growth of *Enteromorpha intestinalis* varied with the magnitude of the different sources in this experiment. Estuarine sediments were more important for the growth of *E. intestinalis* when water column N was low compared with when it was high. This was evident in the differences in algal growth between estuarine sediment and inert sand treatments. Furthermore, the magnitude of the effect of estuarine sediments on macroalgal growth appeared to be related to the nutrient content of the sediments. Overall, growth was greatest when algae were incubated with sediments from the Middle site, which had the highest initial sediment N and P content.

Sediments can be an important nutrient source to macroalgae when water column nutrients are low, which occurs when large amounts of algae deplete the water column of nutrients. Often there is a poor correlation between macroalgal biomass and water column nutrient concentrations (Fong et al. 1998) and water column nutrients in natural systems are often periodically depleted to the low levels obtained in our experiment. Fong and Zedler (2000) found NO_3 and PO_4 values as low as 0.82 and 0.75 μM, respectively, in Famosa Slough, CA, a poorly flushed estuary, during a large macroalgal bloom. In UNB, NO_3 concentrations in poorly-flushed tidal creeks were as little as one-tenth of those in the deeper, well-circulated main channel, and several macroalgal spp. were more abundant in the creeks (Boyle 2002).

When water column N was high, such as in units from the Head, estuarine sediments did not significantly influence *Enteromorpha intestinalis* growth. However, overall *E. intestinalis* growth in these units was low. Salinity in Head waters was 8–10 psu. Prolonged exposure to salinity < 25 psu can significantly reduce the growth of *E. intestinalis* (Kamer and Fong 2000). Therefore, the lack of the effect of sediments on *E. intestinalis* growth may have had less to do with high water column N meeting the algae's nutrient demand than the inhibition of growth due to low salinity. Nutrients from watersheds, however, are usually transported to estuaries via freshwater; high nutrient levels often correlate with low salinity (Valiela et al. 1992). As such, estuarine sediments may not have significant effects on macroalgal growth when salinity is the limiting factor. Sediments may only affect macroalgal growth when salinity or other factors do not inhibit growth.

The influence of water column and sediment nutrients on algal tissue nutrients also varied with the magnitude of the sources. *Enteromorpha intestinalis* tissue N was lowest where water column N was low, such as in units from the Middle and Lower sites, but this effect was mitigated by the contribution of N from estuarine sediments. When water column N was high, the presence of estuarine sediments did not affect tissue N compared with algae grown over inert sand. Low salinity conditions did not appear to impair the ability of algae to remove N from the water column. *E. intestinalis* tissue N levels were greatest overall in units from the Head of the estuary, probably due to the greater supply of N available in the water column. Algae in these units must have derived

Table 4. Soluble reactive phosphorus concentrations (μM) at the end of each week of the experiment. If all samples within a treatment were < 1.61 μM, the notation "BDL" (Below Detection Limit) is used. In samples with values below and above the detection limit, only the latter values are given. Sample size is noted. If $n \geq 3$, mean $\pm$ 1 SE is given. If $n = 2$, the mean is given without SE.

Site	Week 1			Week 2			Week 3		
	Sediment + Algae	Sand + Algae	Sediment Only	Sediment + Algae	Sand + Algae	Sediment Only	Sediment + Algae	Sand + Algae	Sediment Only
Head	BDL	BDL	BDL	BDL	BDL	BDL	2.98 ± 0.78 (n = 4)	4.11 ± 0.28 (n = 4)	3.55 ± 0.76 (n = 3)
Middle	3.23 (n = 1)	3.23 (n = 1)	6.97 ± 0.38 (n = 5)	3.23 (n = 2)	BDL	10.45 ± 2.05 (n = 5)	5.48 ± 0.51 (n = 4)	6.02 ± 1.45 (n = 3)	7.03 ± 1.75 (n = 5)
Lower	3.55 (n = 1)	3.87 (n = 1)	4.13 ± 0.92 (n = 5)	BDL	11.61 (n = 1)	4.11 ± 0.73 (n = 4)	5.56 ± 0.74 (n = 4)	6.88 ± 0.28 (n = 3)	4.45 ± 0.80 (n = 5)

Figure 4. Sediment nitrogen (A) and phosphorus (B) (as % change from initial) from 3 sites in Upper Newport Bay incubated in the presence or absence of *Enteromorpha intestinalis*. Bars represent $\pm$ 1 SE. Two-factor ANOVA summary statistics for nitrogen: site: $p = 0.073$; algae: $p = 0.353$; and for phosphorus: site: $p = 0.001$; algae: $p = 0.186$.

most, if not all, of their N from the water column as tissue N content was the same for treatments with and without estuarine sediments.

Enteromorpha intestinalis tissue P content was greatly enhanced by the presence of estuarine sediments in units from all three sites. Additionally, tissue P was influenced by the magnitude of sediment P availability; algae with the highest tissue P concentration came from units containing sediments from the Middle site, which had the highest initial sediment P value. Similarly, Birch et al. (1981) found that *Cladophora albida* tissue P was tightly linked to sediment P content.

The depletion of water column nutrients in units without macroalgae may be explained by several different processes. Since dissolved nutrients, particularly ammonia, are not known to readily volatilize (Ryther et al. 1981; Lotze and Schramm 2000; Runcie et al. 2003), we assume the nutrients were

sequestered in another compartment of the experimental units. Nutrients may have been taken up by phytoplankton, microphytobenthos, or bacteria, though we did not find visible evidence of significant production by any of these groups in our units. Alternatively, the estuarine sediments in the sediment + water units, which contained no macroalgae, may have been a sink for water column nutrients, particularly NO_3. While NH_4 is often released from estuarine sediments, significant flux of NO_3 into sediments can occur (Boynton et al. 1980; Nowicki and Nixon 1985; Cowan and Boynton 1996; Trimmer et al. 1998, 2000; Magalhães et al. 2002) via diffusive processes when water column NO_3 concentrations greatly exceed porewater NO_3 concentrations (K. Kamer, unpubl. data).

There were no strong indications of loss of N and P from the sediments reflecting the observed increases in *Enteromorpha intestinalis* growth and tissue N and P. Because N and P generally flux from the top layers of sediment (Lavery and McComb 1991; Clavero et al. 2000; Svensson et al. 2000; Trimmer et al. 2000), analyzing only the top layers of the cores may have provided better resolution of changes over time. Alternatively, the mass of nutrients contained within the sediments may have been much greater than the mass of N and P in the algae. While it was possible to detect changes in algal nutrient concentration, concentration changes that may have occurred in sediments might not have been detectable.

Our data provide direct experimental evidence that macroalgae can utilize nutrients stored in estuarine sediments, confirming the long-standing hypothesis that sediments can supply nutrients to primary producers. While many studies have measured and calculated nutrients fluxes from estuarine sediments (e.g., Boynton et al. 1980; Nowicki and Nixon 1985; Cowan and Boynton 1996), only a few studies have investigated whether macroalgae are able to use these sediment-derived nutrients. Lavery and McComb (1991), Thybo-Christesen et al. (1993), McGlathery et al. (1997), and Tyler et al. (2001) provided evidence that macroalgal mats can intercept nutrient flux from sediments. Our study advances the understanding of sediment-macroalgal nutrient dynamics by demonstrating that these nutrients are of ecological significance to the algae by enhancing growth rates and tissue nutrient content. Therefore, it may be important to incorporate nutrient loads from estuarine sediments as well as watersheds into assessments of sources of nutrients to primary producers.

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